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Chronic Exposure of Rats to 100-MHz (CW) Radiofrequency Radiation: Assessment of Biological Effects

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16. ABSTRACT

A multidisciplinary approach was employed to assess the possible biological effects of chronic exposure of rats to 100-MHz continuous wave (CW) radiofrequency (RF) radiation. A group of 20 time-bred rats were exposed in a transverse electronmagnetic mode (TEM) transmission line to 100-MNz at a forward power of 500 W (46 mW/cm^2) starting on Day 6 of gestation under controlled temperature and humidity conditions. Pregnant dams and later their offspring were exposed daily for 4 hr for up to 97 days of age. An equal number of sham-exposed animals, maintained under the same environmental conditions, served as controls. Specific absorption rates (SAR's) for rats of varying ages were determined by twin-well calorimetry. The average SAR for all rats used in the twin-well calorimetry measurements was calculated to be 2.8 ± 1.5 mW/g. ($\bar{x}$ ± SD). Between exposures, animals were evaluated using various developmental and biological indices. No difference was observed between 100-MHz-exposed and sham-exposed rats for complete blood counts, mitrogen-stimulated response of lymphocytes, frequency of T- and B-lymphocytes, or antibody response to Streptococcus pneumoniae capsular polysaccharide. No mutagenic effect on the sperm cells of rats exposed for over 90 days to 100 MHz was observed using the Dominant Lethal Assay. The mean time to eye opening was significantly accelerated in exposed compared to sham-exposed rats; however, no other significant difference in neutrological development was observed. In rats exposed to 100MHz, significant decreases in the activity of acetylocholinesterase were observed in the striatum and medulla oblongata of 22-day-old rats and in the midbrain of 40-day-old rats but not in 97-day-old animals.

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Chronic Exposure of Rats to 100-MHz (CW) Radiofrequency Radiation: Assessment of Biological Effects

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A multidisciplinary approach was employed to assess the possible biological effects of chronic exposure of rats to 100-MHz continuous wave (CW) radiofrequency (RF) radiation. A group of 20 time-bred rats were exposed in a transverse electromagnetic mode (TEM) transmission line to 100 MHz at a forward power of 500 W (46 mW/cm^2) starting on Day 6 of gestation under controlled temperature and humidity conditions. Pregnant dams and later their offspring were exposed daily for 4 hr for up to 97 days of age. An equal number of sham-exposed animals, maintained under the same environmental conditions, served as controls. Specific absorption rates (SAR's) for rats of varying ages were determined by twin-well calorimetry. The average SAR for all rats used in the twin-well calorimetry measurements was calculated to be 2.8 ± 1.5 mW/g ($\bar{x}$ ± SD). Between exposures, animals were evaluated using various developmental and biological indices. No difference was observed between 100-MHz-exposed and sham-exposed rats for complete blood counts, mitogen-stimulated response of lymphocytes, frequency of T- and B-lymphocytes, or antibody response to *Streptococcus pneumoniae* capsular polysaccharide. No mutagenic effect on the sperm cells of rats exposed for over 90 days to 100 MHz was observed using the Dominant Lethal Assay. The mean time to eye opening was significantly accelerated in exposed compared to sham-exposed rats; however, no other significant difference in neurological development was observed. In rats exposed to 100 MHz, significant decreases in the activity of acetylcholinesterase were observed in the striatum and medulla oblongata of 22-day-old rats and in the midbrain of 40-day-old rats but not in 97-day-old animals.

INTRODUCTION

The purpose of this study was to determine if chronic pre- and postnatal exposure of rats to 100-MHz continuous wave (CW) radiofrequency (RF) radiation is of biological consequence. A frequency of 100 MHz was used because the frequency-modulated (FM) radio band is one of the frequency ranges which makes the greatest contribution to public exposure to ambient RF radiation (*1*). This frequency range is of particular interest to the Environmental Protection Agency from the standpoint of assessment of possible health effects and standard setting. Another consideration is the fact that this frequency is very close to the predicted resonant

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frequency (70–80 MHz) for man in free space and is, in fact, resonant for a young child (*2*).

In the resonant frequency region, the predicted whole-body specific absorption rates (SAR's) for human models in free space lie in the range of approximately 2–3 mW/g for a 10 mW/cm² incident power density (*2*). One of the primary objectives of this study was to achieve approximately the same whole-body SAR values in the experimental subjects being irradiated in order to attempt to make a valid comparison with 100-MHz human exposures at 10 mW/cm². The exposure of humans to 10 mW/cm² at 100 MHz, however, occurs only under extreme conditions, such as when a repairman is on an FM antenna tower. The efficiency of absorption is much poorer for rats at 100 MHz than it is for humans owing to the small size of rats relative to the 3-m wavelength. An exposure level approximately five times greater is therefore required to achieve roughly the same SAR values in rats. Consequently, we used an incident power density of 46 mW/cm².

It is not known whether FM broadcast operations pose any threat to the health and well-being of the general population at the power density levels (0.01–0.1 μW/cm²) found in the environment (*1*). The present study attempts to qualitatively estimate the interaction of RF radiation with several biological systems by multidisciplinary evaluation of the biological effects of chronic exposure of rats to 100-MHz radiation. During the course of this study animals were evaluated for various developmental and biological indices which were selected largely because of published reports of biological effects caused by nonionizing electromagnetic radiation (NEMR). For example, immune function was assessed because of reports of changes in the immune system of animals exposed to NEMR (*3–7*). Varma and Traboulay (*8*) reported that exposure of male mice to NEMR causes mutagenic effects. In the present study the mutagenic potential of 100-MHz chronic exposure was assessed using the Dominant Lethal Assay (*9*). Regional brain weights, protein concentration, and acetylcholinesterase activity were determined in the present study in part because of the report of Baranski and Edelwejn (*10*) where alterations in brain cholinesterase activity were found after long-term exposure to NEMR. Finally, growth and neurological development were assessed during the course of this experiment.

MATERIALS AND METHODS

100-MHz Exposure System

The 100-MHz RF radiation exposure system shown in Fig. 1 consists of a rectangular strip transmission line in which a uniform TEM field is propagated. The design is based on criteria reported by Crawford (*11*) and Weil (*12*). The transmission line was constructed using 0.3-cm-thick aluminum sheets. The ground plane separation, b (width), is 76.2 cm. In order to assure a uniform field across the center plate, the width-to-height ratio for the outer conductor was chosen at $a/b = 1.5$. This gives a value for a (height) of 114.3 cm. For a transmission line with a 50-ohm characteristic impedance, the ratio w/b was chosen as 1.16 for $a/b = 1.5$, giving a center conductor width, w, of 88.4 cm. The line operates in the fundamental TEM mode up to a frequency of approximately 105 MHz before the

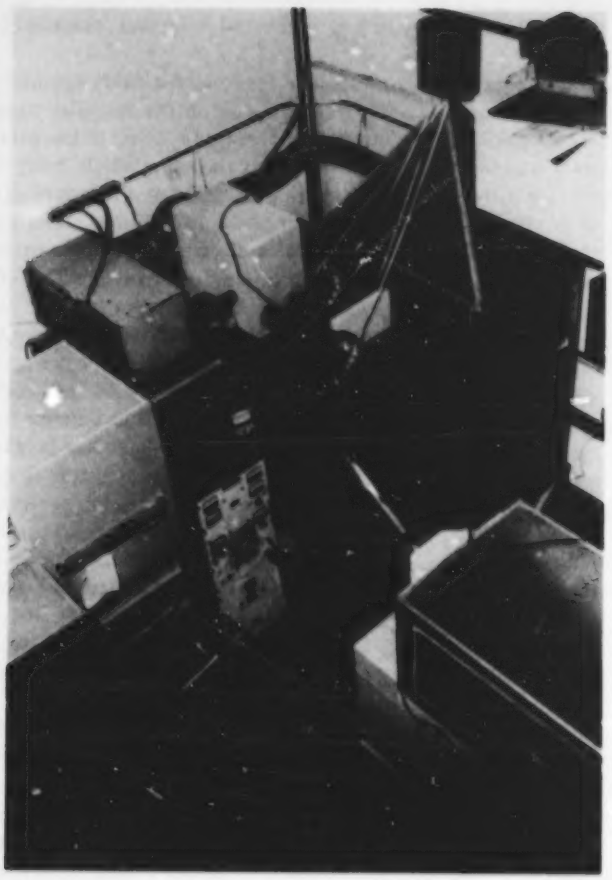

FIG. 1. 100-MHz irradiation chamber (top right) and sham-irradiation chamber (right foreground) as viewed from above. The 1 kW RF amplifier is at left center, beneath the environmental control equipment.

first TE_{01} higher mode begins to propagate (*13*). The length of the transmission line was chosen to be approximately one wavelength long (3.05 m).

The power needed to establish a root mean square (rms) field strength of 194.1 V (10 mW/cm^2) in the middle of the center plate is given by Crawford (*11*):

$$\frac{194.1 \times 0.379^2}{50} = 108.5 \text{ W}.$$

The exposure system is excited by means of a 1 kW RF amplifier unit (model 10270, MCL/Inc., LaGrange, IL) which is driven by a 50-watt RF power generator (model 15022, MCL/Inc.). The system operates at a nominal 500-W output. The RF power

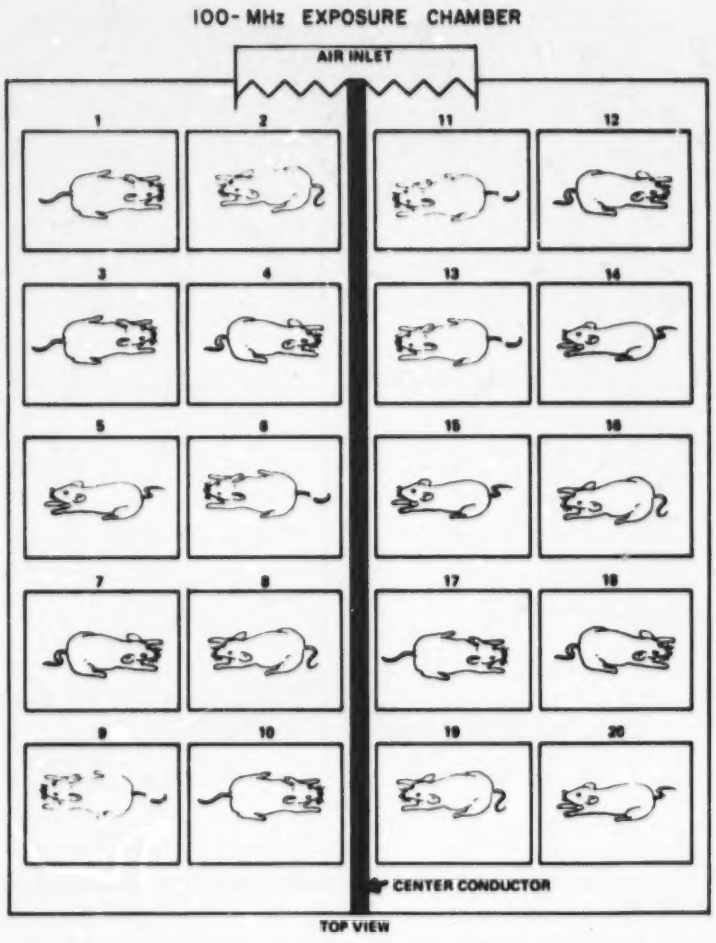

FIG. 2. Plane view of exposure chamber depicting the positioning of cages in relation to center conductor. While rats had ample room for movement within the cages, their orientation was predominantly parallel to the $\vec{E}$ field vector.

is fed through a circulator/isolator (Addington Laboratories, Sunnyvale, CA) and a directional coupler (NARDA Microwave, model 3020A, Plainview, NY) which is used to monitor the incident power to the line, using a thermoelectric power meter (Hewlett–Packard, model 435 A, Palo Alto, CA). The transmission line is terminated with a high power (600 W) load (Bird Electronics, Cleveland, OH).

Since this system was designed to operate in the commercial FM radioband at a relatively high output level, it was important that the equipment operate without excessive leakage of RF energy. This was accomplished by either welding all

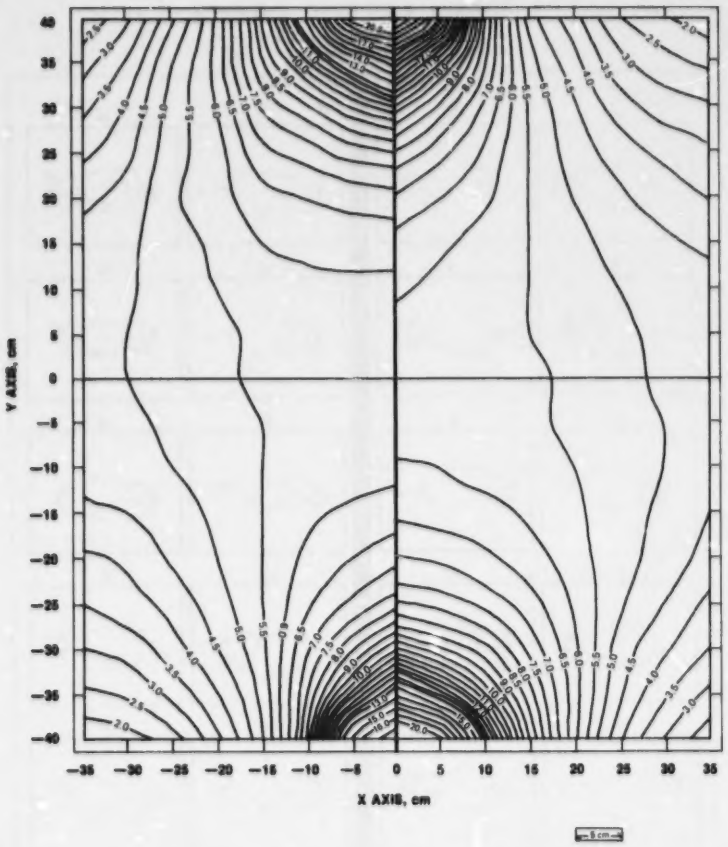

FIG. 3. Cross-sectional field map of Crawford cell exposure area showing contours of constant power density in mW/cm².

joints or using RF gasket material during assembly of the Crawford cell, as well as grounding the structure using a 3.5-cm braided ground strap which led to outside ground rods. Double-shielded coaxial cable (RG-214/U) was also used to connect the power amplifier to the line.

The exposure chamber is provided with two access doors located on the narrow walls (*b* dimension) of the outer rectangle. The doors are made of perforated aluminum to allow for air circulation in the exposure area where animals are located. In addition, perforated aluminum windows covered with 0.3-cm-thick acrylic sheets are located on the wide walls (*a* dimension) in order to allow for illumination of the inside of the chamber. A sham-irradiation chamber which very closely replicates the constructional details of the exposure chamber is located nearby (Fig. 1).

The electric field distribution within the TEM cell was mapped using the fiber-

optically linked EM field probe developed by the Bureau of Radiological Health (*14*) (commercially available from Electronic Instrumentation and Technology Co., Stirling, VA). Measurements were made in the absence of animals in the transverse plane in which test animals were located during irradiation (Fig. 2). The transmission line was operated at a nominal 50-W input level and power density values were recorded at locations every 5 cm along both the X and Y axes in the region where test animals were located (see Fig. 2). Figure 3 shows a contour plot of these measurements; each contour represents a line of constant power density ranging between 2 and 20 mW/cm². Approximately 75% of the cross-sectional area shown is estimated to be within ±20% (±1 db) of the reference value at the center location, $X = \pm 17.5$ cm, $Y = 0$. It is readily apparent from Fig. 3 that enhanced field concentrations do exist in a relatively small region near the edges of the center plate.

Temperature and relative humidity were controlled during irradiations in both the exposure and sham chambers. Cool air from a water-cooled air conditioner was heated electrically to the desired temperature and controlled using a time-proportioning controller as described by Ali (*15*). Steam was then introduced into the mixing chamber to raise the relative humidity to 50%. Relative humidity (RH) was regulated using a 3-mode controller. Two 10-cm insulated ducts distributed the conditioned air to the two chambers. The air was then exhausted into the room through the distal perforated access door. Temperature was sensed with a thermistor probe and relative humidity was sensed with a temperature-compensated Pope-type sulfonated polystyrene ac resistance grid. Both sensors were located in the sham-exposure chamber to minimize the effects of load variability. The airflow measured at the air duct–chamber interface, using a thermoanemometer (Alnor Instrument Co., Niles, IL), was approximately 1.1 m³/min for the 100-MHz exposure chamber and 1.6 m³/min for the sham-exposure chamber. The reason for this difference is not known but may be due to an air-baffling effect caused by several bends in the air duct leading to the exposure chamber. Both temperature and RH were monitored with a dual-channel strip chart recorder during irradiations.

Animal Exposures

Time-bred pregnant Sprague–Dawley rats (CD, Charles River Labs, Wilmington, MA) were confined individually in vented acrylic cages (Fig. 4) during treatment. Rats were irradiated at a forward power of 500 W which is equivalent to an incident power density of 46 mW/cm² at the line center. Daily 4-hr irradiation (between 0800 and 1400 hr) of 20 pregnant dams commenced on Day 6 of pregnancy and continued until parturition. The temperature was maintained at approximately 22°C (range = 21–22°C) and 50% RH (mean range = 45.6–55.3%). On the day of birth litters were randomly normalized to four male pups which were placed in the acrylic cages now subdivided into four sections (Fig. 4). The temperature was maintained at approximately 27°C (mean range = 27–28°C) and 50% RH (mean range = 44.1–54.2%) in an attempt to approximate the nest temperature during the first 14 days of life and then was lowered to 22°C and 50% RH for the duration of the irradiations. Pups were irradiated daily for 4 hr a day. An identical

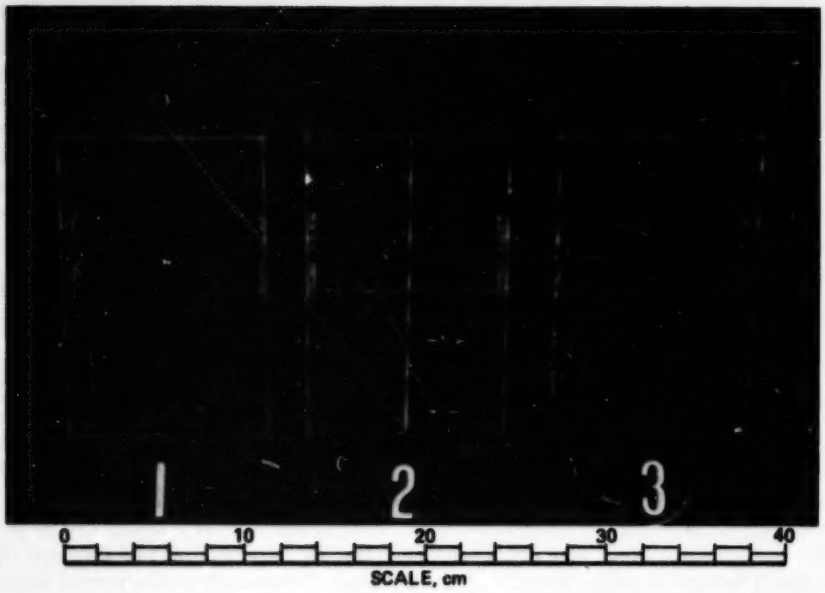

FIG. 4. Vented acrylic cages used to hold rats. (1) One pregnant dam was placed in each cage. (2) At delivery the cage was divided into four sections and was occupied by her pups. (3) At 20 to 22 days of age, half of the pups were removed for tests and the remainder were placed in the cage (divided into two sections) until 40 to 42 days of age when another pup was removed. Thereafter, the remaining pup was held in the unsectioned cage (1). Feces and urine were collected in a tray beneath the cage.

number of control rats was treated concurrently in the sham-exposure chamber. All rats had food and water *ad libitum* prior to and after irradiation. At 20 to 22 days of age, two of the rats in each litter were randomly chosen and removed and used for various tests, as was one pup at 40 to 42 days of age. The remaining pup from each litter was exposed through adolescence (97 days).

Dosimetry

The average specific absorption rates (SAR's) of irradiated rats were determined by twin-well calorimetry (*16*). The SAR measurements were made at a forward power of 500 W for 10 min with no airflow. Measurements of absorption were made on the carcasses of individual animals, which were placed in one of the 20 acrylic cages that were arranged in two rows of five cages per row on either side of the center conductor (Fig. 2). SAR's were determined for rats of various ages and for different positions within the irradiation chamber. The SAR measurements for all but the dams were made using an animal carcass placed in one cage position with the other positions filled with live animals. The positions at which these SAR's were measured were 5, 6, 10, 15, 16, and 19 (Fig. 2). Measurements of SAR's for the dams were not made in the presence of live animals. These determinations were made using carcass pairs in positions 5,16; 6,15; and 10,19.

Body Weight

At selected times during the experiment, rats were weighed as a measure of growth of the pups. Pups were always weighed prior to irradiation. The mean weight of pups in each litter was determined, and the means of these values, i.e., the mean weights of litters of control and irradiated rats, were evaluated by analysis of variance. Data are presented as the mean mass of normalized litters ± 1 SEM and analyzed by analysis of variance.

Developmental Test

Two pups from each litter were tested for the ages at development of startle response, the righting reflex, and the age of eye opening as described by Reiter *et al.* (*17*). The startle response was evoked by a click sounded immediately behind the head of an animal held suspended by the nape of the neck. A positive response consisted of a muscle jerk in any of the extremities. The righting reflex was elicited by holding the rat upside down by the nape of the neck and the tail and dropping it from approximately 30 cm above some wood shavings. A response was considered positive if the animal righted in midair and landed on all four feet. The age at eye opening was recorded with complete opening of both eyes. All tests were performed in the morning prior to exposure. Data are expressed as the mean age of response ±1 SEM and analyzed by analysis of variance.

Locomotor Activity

Locomotor activities of 35- and 84-day-old pups were recorded in a residential maze as described by Reiter *et al.* (*17*). Sixteen irradiated and sham-irradiated rats were tested in the maze for 90 min. Activity counts were recorded over the entire test period using a computer automated minicomputer. Data are expressed as the mean count ±1 SEM and analyzed by analysis of variance.

Hematology

At 22 and 42 days of age, rats were anesthetized with ether and bled from the aorta. Blood smears were prepared with Wright's stain and 200 leukocytes were counted differentially for lymphocytes, macrophages, and polymorphonuclear (PMN) cells. Erythrocyte (RBC) count, leukocyte (WBC) count, mean cell volume (MCV) of erythrocytes, hematocrit (HCT), and hemoglobin (Hb) concentration were evaluated on a Coulter model ZBI-6 counter (Coulter Electronics, Inc., Hialeah, FL). Data are expressed as the mean ±1 SEM.

Immunology

Blood and lymph-node lymphocytes from rats were cultured to determine the mitogen-stimulated response as described by Smialowicz *et al.* (*3*). Triplicate cultures containing 2×10^5 cells in a 0.2-ml volume of RPMI medium (Grand Island Biological Co., Grand Island, NY) with 10% heat-inactivated fetal calf serum, 100 units/ml penicillin, and 100 μg/ml streptomycin were prepared in flat-bottom micro-

titer plates (Falcon Plastics, Oxnard, CA). Several concentrations of the following mitogens, diluted in RPMI medium, were used to stimulate lymphocytes in culture: purified phytohemagglutinin (PHA, Burroughs Wellcome Co., Research Triangle Park, NC), concanavalin A (Con A, Miles Laboratories, Inc., Kankakee, IL), bacterial lipopolysaccharide of *Escherichia coli* 0.28:B12 (LPS, Difco, Detroit, MI), and purified protein derivative of tuberculin (PPD, Parke–Davis and Co., Detroit, MI). Cultures were incubated for 72 hr in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Twenty-four hours before harvesting, cells were labeled with 20 μl of 0.5 μCi of methyl[^{3}H]thymidine (^{3}H-TdR, specific activity 6.7 Ci/m*M*, New England Nuclear, Boston, MA). The cells were collected on glass-fiber filter-paper strips (No. 934AH, Whatman, Inc., Clifton, NJ) with a MASH II automated harvester (Microbiological Associates, Bethesda, MD), and DNA was precipitated with ice-cold 5% trichloroacetic acid. Air-dried samples were transferred to scintillation vials containing Omnifluor–toluene (New England Nuclear) and were counted in a Tri-carb liquid scintillation counter (Packard Instrument Co., Downers Grove, IL).

Considerable variability in the lymphoproliferative response among rats has been observed in this laboratory. To control for this, strict adherence to lymphocyte culture procedures and testing of age- and sex-matched sham-controls at the same time as experimental subjects were done. Data collected as counts per minute (cpm) were $\log_{10}$ transformed to better satisfy the assumption of equality of variance and were analyzed statistically using analysis of variance. Results are given as the arithmetic mean ± 1 SEM.

Complement Receptor and Thymus-Derived Lymphocytes

The number of complement receptor positive (CR^+) cells in rat lymph nodes was determined as described by Bianco *et al.* (*18*) using the 19s fraction of rabbit anti-sheep erythrocyte antibody (Cordis Laboratory, Miami, FL) and fresh mouse serum as a source of complement. The number of cells that formed rosettes (three or more sheep erythrocytes adhering per lymphocyte) was determined. A total of 300 cells each, from duplicate preparations, were counted and the percentage of CR^+ cells was determined.

The percentage of thymus-derived (Θ) cells in rat lymph nodes was determined using a cytotoxicity assay. Lymph-node cells labeled with ^{51}Cr were incubated with rabbit anti-rat thymocyte serum [prepared according to Balch and Feldman (*19*)], followed by guinea pig complement (C′). The cells were sedimented by centrifugation, 100 μl of supernatant was removed, and the radioactivity was measured in a γ-ray spectrometer (Packard Instrument Co.). Total releasable radioactivity was determined by addition of Triton X-100 to an additional group of wells containing labeled cells. Control wells containing labeled cells, normal rabbit serum, and guinea pig complement were prepared simultaneously with the anti-thymocyte serum. The percentage lysis and thus the percentage Θ^+-cells were calculated by the following:

$$\% \text{ specific release} = \frac{X - N}{N - T} \times 100,$$

where X = ^{51}Cr released by cells in the presence of anti-thymocyte serum and C′; N = ^{51}Cr released in the presence of normal rabbit serum + C′; and T = ^{51}Cr released following addition of Triton X-100.

Antibody Response

The primary antibody response to Type-III purified pneumococcal polysaccharide (PPS, a gift from Eli Lilly, Inc., Indianapolis, IN) was measured in weanling rats at 22 days of age. Pups were immunized with a 0.25 μg dose of PPS intraperitoneally. Five days later, rats were bled and serum was collected. Serum antibody titers were determined by microhemagglutination. Titers were analyzed by analysis of variance.

Mutagenic Test

The Dominant Lethal Assay (*9*) was employed to determine if exposure of rats to 100 MHz (CW) caused observable mutagenic changes in sperm cells. At 90 days of age, each sham and irradiated male rat was bred to a pair of normal virgin females for 1 week while the males were continued on their routine schedule of exposure. Eleven days after mating, the females were examined for pregnancy, and the number of live and dead conceptuses, the corpora lutea of pregnancy, and the preimplantation losses were determined. Suspicions of mutagenic effects were based on observation of a significant loss of embryos in the treated group. Analyses were done using χ^2 or analysis of variance where appropriate.

Neurochemistry

At 22, 40, and 97 days of age, rats were decapitated immediately after removal from the exposure chambers. Brains were rapidly removed, placed on ice, and dissected into seven regions as described by Glowinski and Iversen (*20*). These regions were the striatum, hypothalamus, hippocampus, cortex, cerebellum, midbrain, and medulla oblongata. Tissues were weighed, frozen on dry ice, and then stored at −80°C for subsequent analysis. Protein concentration of the brain regions was determined according to Peterson (*21*). Acetylcholinesterase (AChE) activities were determined for all brain regions by the pH stat method of Augustinsson (*22*). Data are expressed as the mean micromoles ACh hydrolyzed per hour per milligram protein ± SEM and were analyzed using Student's unpaired t-test.

RESULTS

Dosimetry

Initial attempts at dosimetric measurements employing forward, reflected, and transmitted powers in the animal-loaded transmission line failed. This was undoubtedly due to the minimal RF interaction between the small load (i.e., rat) and the much longer wavelength (i.e., 3 meters). There was essentially no reflected energy and the transmitted energy was virtually unchanged from the forward power. For this reason, it was decided that the more sensitive method of twin-well calorimetry would be used for measurement of average specified absorption rates.

TABLE I

Specific Absorption Rates (SAR's) of CD Rats Chronically Exposed to 100-MHz (CW) Energy at 46 mW/cm²

Age (days)	*Range of body mass (g)*	*Specific absorption rate ($\bar{x}$ mW/g ± SEM)*
1–10	5–21	2.96 ± 0.31 (32)[a]
21–30	48–97	2.82 ± 0.25 (28)
31–50	110–256	2.55 ± 0.23 (29)
Pregnant dams	278–321	2.02 ± 0.20 (9)

[a] Number of measurements.

Table I shows the SAR values as determined by twin-well calorimetry. SAR's for individual rats were found to be independent of the animal's mass or position in the irradiation chamber for all but the 21- to 30-day-old rats by analysis of variance techniques. For these animals, there was a significant difference in the measured SAR between positions 16 and 10 and positions 10 and 19. The mean SAR for positions 16, 10, and 19 were 1.8, 3.5, and 3.8 mW/g, respectively. This is not

TABLE II

Mean Body Mass of Male Pups Exposed to 100-MHz Energy

Age (days)	*Mean mass of normalized litters[a] ($\bar{x}$ g ± SEM)*	
	Control	*Irradiated*
1	6.56 ± 0.07	6.74 ± 0.09
4	9.50 ± 0.12	9.73 ± 0.15
7	14.8 ± 0.29	15.5 ± 0.27
10	21.3 ± 0.58	22.8 ± 1.27
13	28.3 ± 0.87	30.2 ± 0.85
16	35.3 ± 1.23	38.6 ± 1.23
19	42.0 ± 1.54	45.7 ± 1.32
21	47.7 ± 1.79	51.5 ± 1.51
28	89.7 ± 2.35	97.7 ± 1.99[b]
36	145.0 ± 2.91	150.0 ± 2.68
41	177.0 ± 3.58	177.0 ± 4.92
43	190.0 ± 4.03	198.0 ± 4.43
50	254.0 ± 4.70	263.0 ± 5.88
57	310.0 ± 6.26	325.0 ± 6.04
64	351.0 ± 5.15	365.0 ± 5.82
71	393.0 ± 5.61	400.0 ± 5.59
78	430.1 ± 6.04	435.0 ± 6.26
85	447.1 ± 5.82	452.0 ± 6.04
92	458.1 ± 6.04	465.0 ± 5.82

Note. Masses are expressed as mean values of normalized litters.

[a] Four pups per litter up to 21 days and two pups per litter up to 41 days, thereafter one rat, $N = 20$.

[b] $P < 0.01$, analyses of variance.

TABLE III

Neurological Development and Locomotor Activity Data of Rats Exposed to 100-MHz Energy

	Mean response ± SEM	
	Control	*Irradiated*
Startle response[a] (age in days)	13.2 ± 0.1	13.1 ± 0.1
Eye opening[a] (age in days)	16.0 ± 0.1	15.4 ± 0.1[b]
Righting reflex[a] (age in days)	19.1 ± 0.1	18.8 ± 0.2
Activity at 35 days of age[c] (counts)	1296 ± 106	1226 ± 63
Activity at 84 days of age[c] (counts)	878 ± 49	801 ± 90

[a] Two pups from each of 20 litters tested.
[b] $P < 0.01$, analysis of variance.
[c] One pup from each of 16 litters tested.

surprising since the power density map (Fig. 3) shows a 2-to-1 ratio for positions 16 and 10 and for positions 16 and 19. The failure to observe a significant difference in the SAR values at these different positions for rats of greater or lesser mass can be explained by the variation in SAR values for these animals due to animal-to-animal interactions with the microwave field that offset the expected differences due to the extremes of incident power. The average SAR for all rats used in the twin-well calorimetry measurements was calculated to be 2.77 (SD = 1.45 mW/g) at a forward power of 500 W.

Growth and Development

Table II shows the growth record of pups. Each point up to Day 21 is the mean of the average mass of four pups in either 20 irradiated or 20 sham-irradiated litters. Data for 28 to 41 days are for two pups and thereafter are for one pup per litter. In general, the irradiated pups tended to be larger than sham-irradiated pups. How-

TABLE IV

Peripheral Blood Counts of 42-Day-Old Rats Chronically Exposed to 100-MHz Energy

	Mean ± SEM	
Blood variables	*Control (12)*	*Irradiated (12)*
RBC ($\times 10^{13}$/liter)	5.6 ± 0.2	5.8 ± 0.1
WBC (/mm^3)	7100 ± 550	8000 ± 800
Hb (g/dl)	12.5 ± 0.4	12.7 ± 0.2
MCV (μm^3)	61.8 ± 0.4	61.2 ± 0.8
HCT (mg/dl)	34.5 ± 1.3	35.1 ± 0.6
% PMN	12.9 ± 1.2	13.9 ± 1.7
PMN/mm^3	910 ± 100	1100 ± 170
% Lymphocytes	85.6 ± 1.2	84.7 ± 1.8
Lymphocytes/mm^3	6100 ± 500	6800 ± 700

TABLE V

Mitogen-Stimulated Response of Lymphocytes from Rats Chronically Exposed to 100-MHz Energy

Age (days)	Source of lymphocytes	Mitogen (μg/culture)	[³H]TdR uptake (x̄ CPM ± SEM)		P
			Control (N)	Irradiated (N)	
22	Lymph nodes	0	120 ± 30 (12)	100 ± 20 (12)	N.S.[a]
		PHA-0.5	99,000 ± 12,000	99,000 ± 11,000	N.S.
42	Lymph nodes	0	170 ± 40 (12)	170 ± 20 (12)	N.S.
		PHA-0.5	52,000 ± 8,000	51,000 ± 9,000	N.S.
		ConA-0.5	63,000 ± 10,000	63,000 ± 13,000	N.S.
		PWM-25	1,300 ± 300	1,300 ± 400	N.S.
		LPS-0.5	2,400 ± 400	2,700 ± 500	N.S.
		PPD-25	580 ± 100	640 ± 120	N.S.
42	Blood	0	860 ± 160	1,100 ± 190	N.S.
		PHA-1.0	86,000 ± 9,000	77,000 ± 11,000	N.S.
		ConA-1.0	106,000 ± 13,000	109,000 ± 15,000	N.S.
		PWM-25	8,000 ± 1,600	10,000 ± 2,500	N.S.

[a] Not significant, analysis of variance.

ever, no consistent significant difference was observed in mean body mass between the two groups.

Table III shows the results of tests for neurological development and eye opening. Two pups from each litter were tested for startle response and righting reflex. No differences were observed between irradiated and sham-irradiated pups for development of these responses. Also, no difference was observed in tests for development of locomotor activity at 35 and 84 days of age. A significant difference, however, was observed in the age at eye opening. The mean age at eye opening was almost 1 day later in sham-irradiated rats compared to irradiated rats. This result corresponds to the observation that the exposed rats were slightly larger than sham-irradiates.

Hematology and Immunology

Table IV shows the hemogram record for 42-day-old rats. No difference was observed between irradiated and sham-irradiated rats for the several variables

TABLE VI

Relative Frequency of T- and B-Lymphocytes in Lymph Nodes of 42-Day-Old Rats Exposed to 100 MHz Energy

Rats (N)	Mean ± SEM	
	% CR^+ (B-Cells)	% Θ^+ (T-Cells)
Control (12)	24.7 ± 1.2	29.7 ± 2.9
Irradiated (12)	25.3 ± 1.5	28.8 ± 2.2

TABLE VII

Antibody Titers to *S. pneumoniae*

Rats[a] *(N)*	*Antibody titer (Mean $\log_2$ titer ± SEM)*
Control (14)	3.6 ± 0.2
Irradiated (13)	4.1 ± 0.3

[a] Rats immunized at 22 days of age.

measured, nor in 22-day-old rats (data not shown). Table V shows the *in vitro* lymphocyte response to stimulation by T- and B-cell mitogens. No difference was observed in the response of lymphocytes from irradiated and sham-irradiated rats at 22 and 42 days of age. Table VI shows the relative frequencies of CR^+ and Θ^+ lymphocytes from lymph nodes. These are subpopulations of B- (bone marrow) and T- (thymus-derived) lymphocytes, respectively. No difference was observed between irradiated and sham-irradiated rats. The antibody titers of 22-day-old rats immunized with *S. pneumoniae* are shown in Table VII. No significant difference in response was observed between irradiated and sham-irradiated rats.

Dominant Lethal Test

The Dominant Lethal Assay is an *in vivo* test for germ-cell mutagenesis (*9*). The test is based on the assumption that embryos which are the result of fertilization by genetically altered male germ cells are usually nonviable. The test requires the treatment of the male to some potentially mutagenic agent (in this case, microwaves) followed by the mating of the male to a normal, untreated female. Increased preimplantation death associated with a decreased number of live fetuses would infer a dominant and lethal mutagenesis in the sire's germ cell. In practice, the preimplantation loss is estimated by subtracting the number of live and resorbed fetuses of the pregnancy from the number of corpora lutea (the number of follicles in both ovaries) of that pregnancy. In this experiment, as seen in Table VIII, there

TABLE VIII

Dominant Lethal Assay Results

Variables	*Control*	*Irradiated*
Males	20	20
Females bred to males	36	37
Pregnant females	30	27
% Pregnant	83	73
Live fetuses[a]	13.2 ± 0.7	12.7 ± 0.9
Resorbed fetuses[a]	0.6 ± 0.2	0.8 ± 0.3
Total fetuses[a]	13.8 ± 0.7	13.5 ± 0.8
Preimplantation loss[a]	3.1 ± 0.7	3.0 ± 0.8

[a] Values are means ± SEM, by litter.

TABLE IX

Regional Brain Weights of Rats Chronically Exposed to 100-MHz Energy

TREATMENT	AGE, days	MEAN BRAIN TISSUE WEIGHTS ± SE						
		STRIATUM	HYPOTHALAMUS	HIPPOCAMPUS	CORTEX	CEREBELLUM	MIDBRAIN	MEDULLA
CONTROL	22 (N=10)	0.049 ± 0.002	0.072 ± 0.003	0.109 ± 0.004	0.803 ± 0.007	0.194 ± 0.004	0.125 ± 0.007	0.163 ± 0.004
IRRADIATED	22 (N= 9)	0.048 ± 0.002	0.068 ± 0.001	0.100 ± 0.003	0.797 ± 0.009	0.201 ± 0.008	0.118 ± 0.006	0.155 ± 0.006
CONTROL	40 (N= 9)	0.086 ± 0.003	0.084 ± 0.003	0.130 ± 0.003	0.943 ± 0.017	0.215 ± 0.006	0.170 ± 0.004	0.190 ± 0.005
IRRADIATED	40 (N= 9)	0.051 ± 0.005	0.082 ± 0.004	0.127 ± 0.006	0.942 ± 0.007	0.254 ± 0.005	0.170 ± 0.012	0.228 ± 0.009*
CONTROL	97 (N= 9)	0.089 ± 0.003	0.094 ± 0.003	0.147 ± 0.006	1.061 ± 0.013	0.312 ± 0.008	0.232 ± 0.010	0.275 ± 0.007
IRRADIATED	97 (N= 9)	0.071 ± 0.003	0.097 ± 0.004	0.151 ± 0.005	1.058 ± 0.007	0.317 ± 0.004	0.235 ± 0.005	0.280 ± 0.006

*$P < 0.05$, Student's t-test

was no significant change in preimplantation loss or number of live fetuses in the irradiated group. If no mutagenic effect is seen, the reproductive efficiency of the males can be estimated by observing other fetal variables. There were no statistical differences seen in any of these variables (pregnancy rate, number of live fetuses, total number of fetuses) when sham-irradiated and irradiated groups were compared.

Therefore, we found no evidence of male germ-cell mutagenesis or decreased male reproductive function under these experimental conditions.

Neurochemistry

Regional brain weights of control and exposed rats at 22, 40, and 97 days of age are presented in Table IX. There was a significant elevation of the mean weight of the medulla oblongata in the exposed group at 40 days of age but not at 97 days. No other significant alterations in regional brain weights were seen. Chronic exposure to 100 MHz had no significant effect on regional brain protein concentrations at any age (Table X).

TABLE X

Regional Brain Protein Concentrations in Rats Chronically Exposed to 100-MHz Energy

TREATMENT	AGE, days	REGIONAL BRAIN PROTEIN CONCENTRATIONS, mg protein/gm tissue						
		STRIATUM	HYPOTHALAMUS	HIPPOCAMPUS	CORTEX	CEREBELLUM	MIDBRAIN	MEDULLA
CONTROL	22	73.3 ± 2.7[a] (10)	74.7 ± 4.4 (10)	95.6 ± 8.4 (9)	102.9 ± 6.8 (9)	82.6 ± 9.2 (10)	79.7 ± 8.0 (9)	87.9 ± 9.8 (10)
IRRADIATED	22	73.7 ± 6.6 (9)	80.1 ± 5.5 (8)	109 ± 23.0 (9)	106.0 ± 9.3 (7)	120.9 ± 24.4 (9)	101.6 ± 25.7 (9)	89.9 ± 8.2 (8)
CONTROL	40	96.8 ± 4.1 (8)	101.0 ± 5.2 (9)	109.0 ± 3.3 (8)	118.0 ± 14.0 (7)	121.6 ± 25.0 (8)	94.4 ± 7.6 (8)	98.2 ± 5.8 (8)
IRRADIATED	40	88.9 ± 10.6 (7)	94.9 ± 8.2 (7)	106 ± 7.6 (7)	128.7 ± 28.1 (7)	84.3 ± 12.8 (7)	95.8 ± 8.3 (8)	98.1 ± 8.8 (8)
CONTROL	97	90.0 ± 4.1 (4)	84.8 ± 8.1 (4)	87.0 ± 3.9 (4)	99.0 ± 5.2 (4)	92.8 ± 19.0 (4)	91.3 ± 6.1 (4)	98.0 ± 12.5 (4)
IRRADIATED	97	79.6 ± 12.3 (3)	87.0 ± 7.2 (4)	82.8 ± 6.5 (4)	112.3 ± 15.8 (3)	102.3 ± 7.5 (4)	83.5 ± 3.2 (4)	82.5 ± 3.2 (4)

[a]Mean ± standard error of the mean. Numbers in parentheses refer to n.

TABLE XI

Regional Brain Acetylcholinesterase Activity in Rats Chronically Exposed to 100-MHz Energy

TREATMENT	AGE, days	REGIONAL BRAIN ACETYLCHOLINESTERASE ACTIVITY, μmoles ACh hydrolysed/hr/mg protein						
		STRIATUM	HYPOTHALAMUS	HIPPOCAMPUS	CORTEX	CEREBELLUM	MIDBRAIN	MEDULLA
CONTROL	22	18.2 ± 0.6[a] (5)	7.0 ± 0.5 (5)	3.1 ± 0.5 (4)	4.2 ± 0.5 (5)	4.0 ± 0.7 (5)	6.6 ± 0.3 (4)	6.2 ± 0.5 (5)
IRRADIATED	22	13.7 ± 2.1[b] (3)	7.4 ± 2.1 (4)	3.8 ± 0.7 (4)	4.2 ± 0.2 (3)	3.0 ± 0.9 (4)	6.6 ± 1.6 (4)	6.3 ± 0.3[b] (4)
CONTROL	40	16.2 ± 0.3 (4)	6.5 ± 0.8 (4)	4.2 ± 0.3 (4)	5.4 ± 0.8 (4)	4.2 ± 0.5 (4)	9.2 ± 0.7 (4)	8.0 ± 0.5 (3)
IRRADIATED	40	20.7 ± 4.2 (3)	5.2 ± 0.3 (3)	4.4 ± 0.4 (4)	4.5 ± 0.7 (4)	3.8 ± 0.8 (4)	7.1 ± 0.4[b] (4)	6.4 ± 0.7 (4)
CONTROL	97	19.1 ± 1.4 (4)	7.5 ± 0.7 (4)	5.6 ± 0.3 (4)	7.9 ± 0.6 (4)	2.8 ± 0.6 (4)	8.0 ± 0.9 (4)	6.4 ± 1/2 (4)
IRRADIATED	97	25.5 ± 3.7 (3)	7.0 ± 0.4 (4)	5.8 ± 0.5 (4)	6.8 ± 1.3 (3)	2.7 ± 0.3 (4)	7.1 ± 0.3 (4)	6.7 ± 0.4 (4)

[a]Mean ± standard error of the mean. Numbers in parentheses refer to n.

[b]$P < 0.05$, Student's t-test

Regional brain AChE activities are presented in Table XI. Striatal and medulla AChE activities were significantly depressed at 22 days in the exposed group. Midbrain AChE activities in exposed rats were significantly lower than control activities at 40 days of age.

DISCUSSION

Chronic pre- and postnatal exposure of rats to 100-MHz (CW) RF radiation resulted in a slight increase in growth, acceleration in the development of eye opening, and transient alterations in regional brain AChE activities. On the other hand, no changes in neurological development or the hematologic, immunologic, or reproductive systems of these animals were observed.

The tentative results of acceleration in the development of eye opening require further investigation. The mean age at eye opening was almost 1 day later in sham-irradiated compared to irradiated rats. This result corresponds to the observation that the irradiated rats were slightly larger (greater mass) than sham-irradiates. This trend in body weight differences was observed from birth and continued through the period of observation. For example, on Day 16 irradiated rats that were observed for eye opening weighed 39.6 ± 5.3 g while shams weighed 34.9 ± 5.4 g, $P < 0.02$. These results on first analysis suggest that the exposed rats are developing slightly faster than shams. However, examination of results from other experiments where this test was employed indicates quite another explanation. Reiter *et al.* (*17*) report that development of eye opening occurs on about Day 15 in untreated CD rats. In the present experiment, however, the sham-irradiated rats showed a mean period to eye opening of 16 days. The reason for this delay in eye opening in the sham-irradiated rats is not known. This difference in physical development, body mass, and time to eye opening is not related to any change in neurological development of startle or righting reflex or in locomotor activity of irradiated rats.

No alteration in circulating blood cells was observed for rats 22 and 42 days of age. These results are similar to those reported by Smialowicz *et al.* (*3*) where no change was observed in hemograms of rats chronically exposed to 2450 MHz. There was no difference between irradiated and sham-irradiated rats for antibody response to *S. pneumoniae*, mitogen-stimulated response of lymphocytes, and frequency of T- and B-lymphocytes in lymph nodes. From these results it appears that the immune system of rats chronically exposed to 100 MHz under the conditions described is unaffected.

In the present study, no mutagenic effect on the sperm of irradiated rats was observed using the Dominant Lethal Assay (*9*).

AChE activities were significantly depressed only in the striatum and medulla of 22-day-old irradiated rats and in the midbrain of 40-day-old rats. In partial agreement with the results reported here, Baranski and Edelwejn (*10*) reported a loss of active AChE centers in the rabbit cortex, diencephalon, mesencephalon, metencephalon, and cerebellum after repeated exposure (2950 MHz, 5 mW/cm², 2 hr daily for 3 or 4 months) to microwaves. The most pronounced effects were observed in the diencephalon and mesencephalon which generally correspond to the hypothalamic and midbrain regions studied here. Olcerst and Rabinowitz (*23*) suggested that the depression of AChE activity found *in vivo* is not the direct effect of microwave radiation, since in their *in vitro* system microwave radiation affected enzyme activity only when the temperature increase was great enough to permanently denature the enzyme. Taking this into consideration, the transient depressions in regional brain AChE activity reported here are not unexpected and, as suggested by Olcerst and Rabinowitz (*23*), may be the result of alterations in local cation concentrations. In any event, because of the small number of animals assayed for this enzyme, it is necessary to examine this and other neurochemical parameters before definitive conclusions concerning possible neurobiological effects of chronic 100-MHz exposure are formulated.

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